Biochimica et Biophysica Acta, 644 (1981) 153–156
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71520

HYDROFLUORIC AND NITRIC ACID TRANSPORT THROUGH LIPID BILAYER MEMBRANES

JOHN GUTKNECHT * and ANNE WALTER

Department of Physiology, Duke University Medical Center, and Duke University Marine Laboratory, Beaufort, NC 28516 (U.S.A.)

(Received December 18th, 1980)

Key words: Hydrofluoric acid; Nitric acid; Membrane permeability; Phospholipid; Cholesterol; Acid rain

Hydrofluoric and nitric acid transport through lipid bilayer membranes were studied by a combination of electrical conductance and pH electrode techniques. Transport occurs primarily by nonionic diffusion of molecular HF and HNO_3. Membrane permeabilities to HF and HNO_3 ranged from 10^{-4} to 10^{-3} cm · s^{-1}, five to seven orders of magnitude higher than the permeabilities to NO_3^-, F^- and H^+. Our results are consistent with the hypothesis that F^- transport through biological membranes occurs mainly by nonionic diffusion of HF. Our results also suggest that of the two principal components of 'acid rain', HNO_3 may be more toxic than H_2SO_4.

Transport and accumulation of inorganic acids and their conjugate bases are important in toxicology and physiology, but the mechanisms of most of these processes are poorly understood. For example, nitric and sulfuric acids are the principal components of 'acid rain' which is highly toxic to aquatic plants and animals [1,2]. Although the mechanisms of toxicity are unknown, a reasonable hypothesis is that toxicity is related to the rates at which the protonated (nonionic) forms of the acids diffuse through cell and epithelial membranes, e.g., gills. However, no information is available on the membrane permeabilities of these acids through cell membranes and epithelia. Secondly, fluoride addition to public water supplies has prompted studies on the mechanisms of F^- transport and accumulation in biological systems. Based on the pH dependence of F^- transport, several investigators [3–6] have suggested that F^- crosses cell membranes mainly in the nonionic form, i.e., HF. However, there is no direct evidence for this idea and little information on the permeabilities to F^- and HF.

The lipid bilayer region of biological membranes is the principal barrier to the passive diffusion of water and most solutes. Thus, synthetic lipid bilayers are useful models for estimating the permeability of any solute which interacts primarily with membrane lipids. For the study of acid/base transport, lipid bilayers are advantageous because electrical and concentration gradients can be easily controlled, and one can utilize large pH gradients which would alter the permeability of biological membranes which contain large amounts of protein. In the present study we used a combination of electrical conductance and pH electrode techniques to measure the permeabilities of nitric, hydrofluoric, sulfuric and phosphoric acids through planar lipid bilayers made of egg lecithin or egg lecithin plus cholesterol.

Lipid bilayers were formed by the brush technique [7] on a 1.8 mm^2 hole in a polyethylene partition which separated two aqueous solutions of 1.1 ml each. Both solutions were stirred magnetically, and the front solution was perfused continuously with various solutions at a rate of about 1 ml · min^{-1}. The rear compartment contained unbuffered NaCl (50

* To whom correspondence and reprint requests should be addressed at: Duke University Marine Laboratory, Pivers Island, Beaufort, NC 28516, U.S.A.

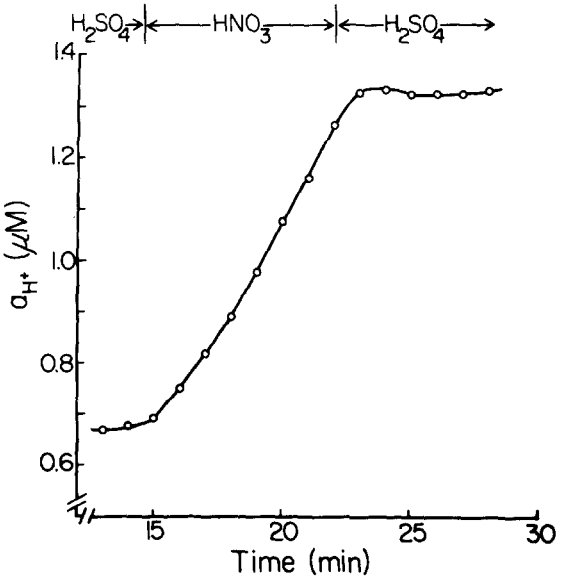

Fig. 1. Net proton flux produced by a gradient of HNO_3 across a lipid bilayer membrane. The H^+ activity (a_{H^+}) was measured with a small pH electrode in the rear compartment which contained unbuffered NaCl (50 mM). The front compartment was perfused with either H_2SO_4 (200 mM, pH 1.0) or HNO_3 (130 mM, pH 1.0). The membrane potential was clamped at 0 mV.

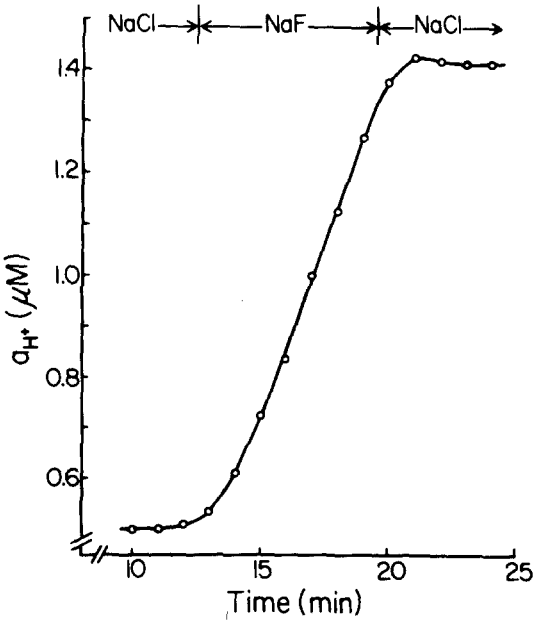

Fig. 2. Net proton flux produced by a gradient of NaF across a lipid bilayer membrane. The rear compartment contained unbuffered NaCl (50 mM), and the front compartment was perfused with either NaCl (20 mM, pH 4.1) or NaF (20 mM, pH 4.1). The front compartment also contained sodium citrate buffer, 30 mM. The membrane potential was clamped at 0 mV.

mM) and a small combination pH electrode (Markson, Model 583). Both front and rear compartments also contained calomel-KCl electrodes, which allowed measurements of the membrane voltage and conductance. All solutions were equilibrated with argon, and an argon atmosphere was maintained above the solutions to minimize the absorption of CO_2. The temperature was 24 ± 1°C.

Egg lecithin was obtained from Lipid Products (Surrey, U.K.). Cholesterol, decane and tetradecane were obtained from Sigma Chemical Co. (St. Louis, MO).

Fig. 1 shows the rate of change of hydrogen ion activity (a_{H^+}) in the rear solution when the front compartment is perfused with either H_2SO_4 (200 mM, pH 1.0) or HNO_3 (130 mM, pH 1.0). Fig. 2 shows the rate of change in a_{H^+} in the rear solution when the front solution is either NaCl (20 mM, pH 4.1) or NaF (20 mM, pH 4.1). In both experiments the membrane was formed from a solution of egg lecithin (50 mg/ml) plus cholesterol (25 mg/ml) in tetradecane. The net H^+ flux is equal to the slope times the volume of the rear solution divided by the surface

TABLE I

PERMEABILITY COEFFICIENTS OF HNO_3, HF, NO_3^-, F^- AND H^+ THROUGH LIPID BILAYER MEMBRANES

The membrane composition was either egg lecithin plus cholesterol (1 : 1 mol ratio) in tetradecane or egg lecithin in decane. HNO_3 and HF permeabilities were measured by the pH electrode technique. NO_3^-, F^-, and H^+ permeabilities were estimated from membrane conductances and ionic transference numbers. Results are quoted as: mean ± S.D. (number of membranes).

Solute	Membrane composition	Permeability coefficient ($cm \cdot s^{-1}$)	
HNO_3	Lecithin + cholesterol	$(4.4 \pm 0.8) \cdot 10^{-4}$	(5)
HNO_3	Lecithin	$(9.2 \pm 1.5) \cdot 10^{-4}$	(7)
HF	Lecithin + cholesterol	$(1.4 \pm 0.3) \cdot 10^{-4}$	(5)
HF	Lecithin	$(3.1 \pm 1.4) \cdot 10^{-4}$	(7)
NO_3^-	Lecithin + cholesterol	$(1.3 \pm 0.6) \cdot 10^{-10}$	(2)
F^-	Lecithin + cholesterol	$(4.9 \pm 2.3) \cdot 10^{-11}$	(2)
H^+	Lecithin + cholesterol	$(1.7 \pm 0.8) \cdot 10^{-9}$	(2)

area of the membrane. From the pK values of these acids ($pK_{HNO_3} = -1.3$, $pK_{HF} = 3.1$) and the activity coefficients of their salts [8–11] we can calculate the concentrations of molecular HNO_3 and HF in the aqueous solutions. From these values we can estimate the permeability coefficients (flux/concentration gradient), i.e., $P_{HNO_3} = 4.4 \cdot 10^{-4}$ cm · s^{-1} and $P_{HF} = 1.4 \cdot 10^{-4}$ cm · s^{-1} (Table I).

In the pH electrode experiments the membrane voltage was clamped at zero. Due to electrical noise from the pH meter, we measured the zero-potential currents in separate experiments. Under the conditions shown in Figs. 1 and 2, the net current (expressed as moles of monovalent ion per cm^2 per s) was always less than 1% of the net H^+ flux, which suggests that the net H^+ flux occurs by an electrically silent mechanism, i.e., diffusion of HNO_3 or HF. We also found that the net H^+ flux was proportional to the first power of the concentration of either HNO_3 or HF, which indicates that HNO_3 or HF monomers are diffusing through the membrane.

We also measured the permeabilities to NO_3^-, F^- and H^+. From the ionic transference numbers and membrane conductances, the one-way fluxes and permeabilities can be calculated [12]. As expected, the ionic permeabilities are five to seven orders of magnitude lower than the permeabilities to HNO_3 and HF (Table I). When $P_{NO_3^-}$ was measured in HNO_3 (pH = 1.0), the value obtained was about 10-fold higher than the value shown in Table I, presumably because at low pH the egg lecithin becomes positively charged [13]. P_{F^-} was not measured at low pH because high conentrations of HF damage the glass electrodes.

No detectable H^+ flux was observed when the front solution was H_2SO_4 (200 mM) (Fig. 1). From the dissociation constants and activity coefficients of H_2SO_4 and HSO_4^- [9,11], we estimate the molecular H_2SO_4 activity to be $1.6 \cdot 10^{-5}$ M. Since the smallest net H^+ flux we can measure with the pH electrode is about $2 \cdot 10^{-12}$ mol · cm^{-2} · s^{-1}, we estimate that the H_2SO_4 permeability is less than 10^{-4} cm · sec^{-1}. Using a similar technique, we have estimated the permeability to phosphoric acid to be less than $6 \cdot 10^{-8}$ cm · s^{-1}.

Our results indicate that both HNO_3 and HF are highly permeant solutes with permeability coefficients similar to that of water [14]. The pH electrode and lipid bilayer techniques can also be used to measure permeabilities to other small acids and bases which are difficult or impossible to study in biological membranes. For example, the molecular HCl permeability of lipid bilayers is about 3 cm · s^{-1}, nine orders of magnitude higher than the permeabilities to H^+ or Cl^- [15].

The HNO_3 and HF permeabilities of egg lecithin-decane bilayers are about 2-fold higher than the permeabilities of egg lecithin-cholesterol-tetradecane bilayers (Table I). The higher permeabilities may be due to the larger area per molecule (increased fluidity) [14], the higher mole fraction of hydrocarbon solvent in the membrane [16], and/or the higher partition coefficient of HNO_3 and HF into the hydrocarbon region of the membrane [19].

Our results are consistent with the hypothesis that F^- transport through biological membranes occurs primarily by nonionic diffusion of HF [3–6]. If this is true, then the equilibrium distribution of F^- can be used to estimate intracellular pH [4–6]. The observed similarities between intracellular pH values estimated from F^- distribution and the distribution of other permeant weak acids and bases has been recently cited as evidence for nonionic transport of HF in various cells and tissues [4–6]. However, the validity of this argument depends upon the relative permeabilities of biological membranes to HF and F^-. At physiological pH, the ratio of [HF] to [F^-] is less than 10^{-4}. Thus, if the primary transport mechanism is nonionic diffusion of HF, then the ratio P_{HF}/P_{F^-} must be greater than 10^4. Although this may be true for some cells and tissues, it is certainly not true in all cases. For example, P_{F^-} in mammalian erythrocytes is about 10^{-5} cm · s^{-1} [17], only about one order of magnitude lower than P_{HF} for lecithin-cholesterol bilayers (Table I). Thus, the fact that the F^- distribution ratio in erythrocytes provides an accurate estimate of intracellular pH [5] is fortuitous in the sense that it does not depend upon HF permeation. Furthermore, the F^- distribution ratio will reflect intracellular pH in any cell which does not actively transport F^- and H^+, simply because both F^- and H^+ are at electrochemical equilibrium across the membrane, regardless of whether HF diffusion is the primary transport process. Thus, we believe that this particular argument [4–6] for HF transport is inconclusive in most cases. However, the observed pH dependence of F^- transport remains as strong evidence for non-

ionic diffusion of HF, especially at pH <6 [3–6,18].

Finally, our results suggest that of the two primary components of 'acid rain', HNO_3 may be more toxic than H_2SO_4. This would be due to both the higher permeability and much higher concentration of molecular HNO_3 in any solution containing equal concentrations of both acids. If HNO_3 is, in fact, more toxic than H_2SO_4, it will suggest that entry of the nonionic form of the acid is important in toxicity. However, if HNO_3 and H_2SO_4 show similar toxicities, it will suggest that toxicity is due primarily to a low environmental pH and consequent disruption of normal ionic exchange processes across the gills or skin of fresh water animals.

This work was supported by USPHS grants HL 12157 and ES 01908. We thank Dr. G.M. Whitford for reviewing the manuscript.

References

1 Schofield, C.L. (1976) Ambio 5, 228–230
2 Likens, G.E., Wright, R.F., Galloway, J.N. and Butler, T.J. (1979) Sci. Am. 241, 43–51
3 Whitford, G.M., Pashley, D.H. and Reynolds, K.E. (1977) Am. J. Physiol. 232, F10–F15
4 Whitford, G.M., Pashley, D.H. and Reynolds, K.E. (1979) Am. J. Physiol. 236, F141–F148
5 Eisenberg, A.D. and Marquis, R.E. (1980) J. Dent. Res. 59, 1187–1191
6 Armstrong, W.D. and Singer, L. (1980) Proc. Soc. Exp. Biol. Med. 164, 500–506
7 Mueller, P. and Rudin, D.O. (1969) Curr. Top. Bioenerg. 3, 157–249
8 Lange's Handbook of Chemistry (1973) (Dean, J.A., ed.), McGraw Hill Book Co., New York
9 Perrin, D.D. (1969) Dissociation Constants of Inorganic Acids in Aqueous Solution, Butterworth, London
10 Patel, P.R., Moreno, E.C. and Patel, J.M. (1971) J. Res. Natl. Bur. Stand. 75A, 205–211
11 Robinson, R.A. and Stokes, R.H. (1970) Electrolyte Solutions, Butterworth, London
12 Hodgkin, A.L. (1951) Biol. Rev. (Cambridge) 26, 339–365
13 Ohki, S. (1969) J. Coll. Int. Sci. 30, 413–420
14 Finkelstein, A. and Cass, A. (1967) Nature (London) 216, 717–718
15 Gutknecht, J. and Walter, A. (1981) Biochim. Biophys. Acta 641, 183–188
16 Haydon, D.A., Hendry, B.M., Levinson, S.R. and Rquena, J. (1977) Nature (London) 268, 356–357
17 Tosteson, D.C. (1959) Acta Physiol. Scand. 46, 19–41
18 Whitford, G.M., Schuster, G.S., Pashley, D.H. and Venkateswarlu, P. (1977) Infect. Immun. 18, 680–687
19 Simon, S.A. and Gutknecht, J. (1980) Biochim. Biophys. Acta 596, 352–358